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A Comparison of 2-part and 3-part Nanoparticle-Based Sensor

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ABSTRACT

There has long been a drive to produce sensors with ever-increasing sensitivity and selectivity, while also achieving robustness and ease of use. Nanoparticle-based sensing approaches have generated a great deal of attention and excitement, because they possess such qualities. For these assays to function properly, it requires the integration of molecular recognition motifs and materials with outstanding optical properties. Aptamers are DNA or RNA sequences that bind analytes with high specificity, which makes them a suitable choice as recognition elements. Changes in the surface plasmon resonance (SPR) of gold nanoparticles (AuNPS) as a function of interparticle distance, has been used as an optical signal to detect the presence of different species in solution by the naked eye. In this work, we coated gold nanoparticles with short oligonucleotides and aptamers for the design of sensors that can be used under different conditions, including salt concentration, pH and temperatures. Three aptamer sensors were developed using this approach 1) riboflavin, as a general indicator of biological activity, 2) ricin, a toxin that is of broad interest, and 3) theophylline, an adenosine antagonist. Our designs are based on two approaches, the first method consisted of the use of two sets of AuNPs, each coated with a short oligonucleotide complementary to a different part of the sequence of the aptamer of interest. Hybridization of the DNA-coated particles (DNA-AuNPs) with the free aptamer produced aggregates, ***i.e.*** 3-part design. The second approach consisted of the use of only two sets of DNA-AuNPs, one coated with an aptamer that contains a thiol group in its 5´ end, and the second set of AuNPs coated with a sequence complementary to part of the aptamer. Hybridization of these two sets of particles produced aggregates, ***i.e.*** 2-part design. In both cases, the presence of the analyte promoted a change in the conformation of the aptamer, which caused the dehybridization of the complementary sequences. This conformational change of the aptamer upon binding of the analyte produced the dissociation of the nanoparticle aggregates, which is translated into a change in the color of the suspensions from blue to red. In this presentation, we will compare the advantages and disadvantages associated with a 3-part versus a 2-part nanoparticle-oligonucleotide reporting assay.

INTRODUCTION

Gold nanoparticles have been utilized successfully in the design of different kinds of detection systems [1]. Events that affect the surface plasmon resonance of AuNPs produce colorimetric changes in their suspensions, giving a response that can be qualitatively detected by the eye and quantified with simple instrumentation. The design of an effective sensor based on AuNPs can be achieved by the use of aptamers, which are oligonucleotides that bind a specific molecule by changing their conformation to form an optimal binding pocket. Liu and Lu [2]

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14. ABSTRACT

There has long been a drive to produce sensors with ever-increasing sensitivity and selectivity, while also achieving robustness and ease of use. Nanoparticle-based sensing approaches have generated a great deal of attention and excitement, because they possess such qualities. For these assays to function properly, it requires the integration of molecular recognition motifs and materials with outstanding optical properties. Aptamers are DNA or RNA sequences that bind analytes with high specificity, which makes them a suitable choice as recognition elements. Changes in the surface plasmon resonance (SPR) of gold nanoparticles (AuNPs) as a function of interparticle distance, has been used as an optical signal to detect the presence of different species in solution by the naked eye. In this work, we coated gold nanoparticles with short oligonucleotides and aptamers for the design of sensors that can be used under different conditions, including salt concentration, pH and temperatures. Three aptamer sensors were developed using this approach 1) riboflavin, a general indicator of biological activity, 2) ricin, a toxin that is of broad interest, and 3) theophylline, an adenosine antagonist. Our designs are based on two approaches, the first method consisted of the use of two sets of AuNPs, each coated with a short oligonucleotide complementary to a different part of the sequence of the aptamer of interest. Hybridization of the DNA-coated particles (DNA-AuNPs) with the free aptamer produced aggregates, i.e. 3-part design. The second approach consisted of the use of only two sets of DNA-AuNPs, once coated with an aptamer that contains a thiol group in its 5́ end, and the second set of AuNPs coated with a sequence complementary to part of the aptamer. Hybridization of these two sets of particles produced aggregates, i.e. 2-part design. In both cases, the presence of the analyte promoted a change in the conformation of the aptamer, which caused the dehybridization of the complementary sequences. This conformational change of the aptamer upon binding of the analyte produced the dissociation of the nanoparticle aggregates, which is translated into a change in the color of the suspensions from blue to red. In this presentation, we will compare the advantages and disadvantages associated with a 3-part versus a 2-part nanoparticle-oligonucleotide reporting assay.

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designed a 3-piece detection system composed of DNA-AuNPs. The oligonucleotides were complementary to different parts of the aptamer. Hybridization of the DNA-AuNPs with the free aptamer produced aggregates that dissociated upon analyte addition. Changes in the aptamer conformation upon the binding event affected the SPR of the conjugated particles, producing a colorimetric response. In this contribution, we designed a 3-piece sensor for three different analytes: theophylline, riboflavin and ricin. Subsequently, we explored the design a simpler, 2-piece sensor. We coated one set of AuNPs directly with the aptamer through a thiol group at its 5´ end, and a different set of particles with a short DNA sequence, complementary to part of the immobilized aptamer. We studied the conditions necessary for the hybridization of the DNA-AuNPs and analyte detection.

EXPERIMENTAL

Gold nanoparticles (13 nm) were synthesized following procedures from the literature [3]. Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Ricin (from RCA_{60}) was obtained from Vector Laboratories (Burlingame, CA). The aptamer sequences were adapted from the literature [4, 5, 6]. Theophylline, riboflavin and tris(2-carboxyethyl)phosphine hydrochloride (TCEP), were purchased from Aldrich (St. Louis, MO). The oligonucleotides were activated prior to reaction with the AuNPs by exposing them to a 10 mM solution of TCEP in 500 mM acetate buffer. After activation, the nanoparticles (3 mL, 10 nM) were mixed with the oligonucleotides (13.5 μL, 1mM) and left in the dark overnight. Subsequently, tris acetate buffer and NaCl were added to obtain a final concentration of 5 and 100 mM, respectively. The suspensions were stored overnight in the dark. To remove unbound oligonucleotides, the DNA-AuNPs were centrifuged two times at 16 000g and resuspended in 25 mM tris acetate buffer containing 100 mM NaCl. A final centrifugation was performed followed by resuspension in hybridization buffer. The 3-piece aggregates were obtained by mixing the DNA-AuNPs (600 μL each, 13.5 nM) with the free aptamer (18 μL, 10 μM) in hybridization buffer at pH 8.2 and stored at 4 °C. The 2-piece aggregates were obtained by mixing the DNA-AuNPs (600 μL, 13.5 nM) in hybridization buffer and stored at 4 °C. The size of the DNA-AuNPs was measured by dynamic light scattering (DLS) and the surface charge by Zeta potential, in a Zetasizer Nano Instrument (Malvern Instruments Ltd, Worcestershire, UK). The AuNPs were resuspended in Millipore water and the measurements performed at 25 °C. Melting curves were measured in a Cary 300 UV-vis spectrophotometer (Varian Inc, CA). The absorption of the suspensions was monitored at 260 nm at 2 °C intervals. Analyte detection was started by equilibrating 85 μL (10 nM AuNPs) of the suspensions at the temperature of detection for 15 minutes. Subsequently, various volumes of a stock solution of the analyte were added to the aggregate suspensions and the absorption of the mixtures measured after 2 minutes.

DISCUSSION

DNA-AuNPs were obtained by immobilization of the oligonucleotides on the surface of the particles through chemisorption of a thiol group at one end of the DNA sequence. The DNA-AuNPs were characterized by DLS and Zeta-potential, as shown in Table I. The hydrodynamic diameter of the citrate-coated AuNPs is 15 nm. Immobilization of an oligonucleotide composed

of 12 bases (AuNP-DNA12) increased their size by approximately 4 nm, while a 34-base aptamer (AuNP-Aptamer) increased by 15 nm. The size of AuNPs coated with poly(ethylene glycol) (MW= 5000g/mol) was measured as a control. These results indicated a successful coating of the nanoparticles with the thiolated molecules. The surface charge of the different nanoparticles prepared was determined by Zeta-potential measurements. Table I shows that the surface charge of the particles increased when longer oligonucleotides were used. Contrary to the DNA-AuNPs, particles coated with PEG showed a very low electrophoretic mobility, due to the lack of charges on their surface.

Table I. Size analysis and Z-potential values for gold nanoparticles coated with different molecules

	AuNP	AuNP-DNA12	AuNP-Aptamer	AuNP-PEG_{100}
d (nm)	15	19	31	46
Z (mV)	-33.5	-35.6	-41.2	-0.118

The design of the 3-piece sensor required two sets of AuNPs, each one coated with a short sequence complementary to different parts of the free aptamer. The sensor was assembled by combining equimolar amounts of the two sets of particles (as determined by UV absorption) with the aptamer. The mixture was stored overnight at 4 °C to promote the hybridization of the complementary sequences. After approximately 12 h, a solid deposited at the bottom of the vial. To remove excess aptamer, the aggregates were centrifuged at 800g for 90 seconds and the pellet was resuspended in fresh hybridization buffer. The melting curves were obtained by diluting the aggregates suspensions with 25 mM tris acetate buffer (pH 8.2) to obtain the desired NaCl concentration. The Mirkin group has studied the effect of salt concentration upon the hybridization of DNA-AuNPs [7]. They have identified that a sharp melt is observed as a consequence of the cooperative disassembling of the particles upon reaching the melting temperature. The sharpness of the melting curve is affected by various parameters, including a homogeneous coating of the particles and an optimal aptamer loading. The melting curves for the 3-piece theophylline sensor (3-piece-TH) suspended in different concentrations of NaCl are shown in Figure 1A. In all the cases, sharp curves were observed and, as expected, the melting temperature decreased when the salt concentration was reduced. The melting curves for the 3-piece-TH sensor prepared on three different days illustrate the reproducibility of their preparation (Figure 1B).

Analyte detection was performed at temperatures slightly below the point at which the intensity of the absorbance at 260 nm started to increase in the melting curves. This assured a low signal from the sensor before the analyte was added. In all cases, the sensors were left equilibrating for 15 minutes at the temperature of detection. It was observed that during this period, the aggregates disassemble to a certain extent, changing the absorption maxima and intensity. Subsequently, small aliquots of the analyte were added, the suspensions were homogenized after one minute and the absorbance was recorded after one more minute. The process was repeated until the absorption of the suspension did not change or started to decrease. Figure 2A shows the typical response of a 3-piece-TH sensor suspended in 120 mM NaCl at 29 ºC. A quantitative response of the sensor is obtained by plotting the ratio between the absorption

maxima and the background (taken at 700 nm) at different analyte concentrations (Figure 2B). This plot showed that the sensor offered a linear response over a wide range of theophylline concentrations (between 0.5 and 25 µM). The 3-piece sensor designed for ricin (3-piece-RC) showed a superior sensitivity but a narrower detection range (Figure 2C). Current experiments suggested that this can be improved by changing the length and sequence of the oligonucleotides

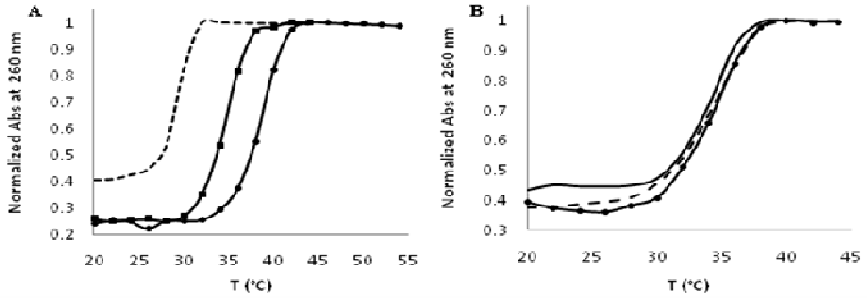

Figure 1. A) Melting curves of the 3-piece-TH sensor prepared in hybridization buffer (300 mM NaCl, 25 mM tris acetate, pH 8.2) and resuspended in 25 mM tris acetate with a concentration of NaCl of: 90 mM (dashed line), 120 mM (squared line) and 150 mM (circled line). B) Melting curves of three different batches of 3-piece-TH sensors resuspended in 120 mM NaCl.

used.

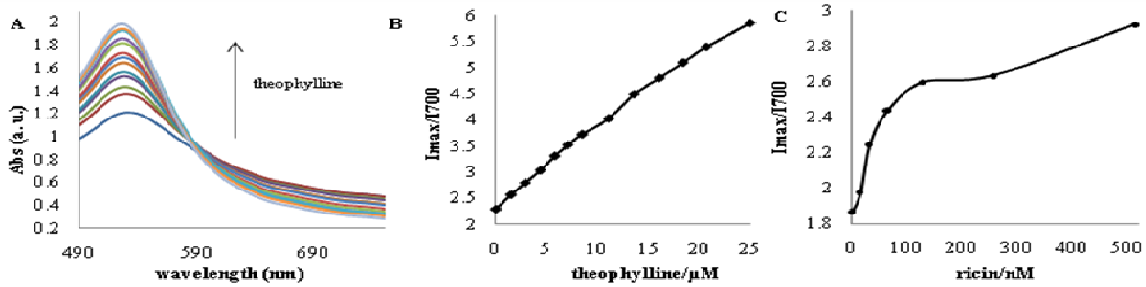

Figure 2. A) Absorbance spectra of 3-piece-TH sensor at various concentrations of theophylline. Plot of the corrected absorption maxima of: B) 3-piece-TH sensor at different concentrations of theophylline and C) 3-piece-RC sensor at different ricin concentrations.

To simplify the design of the sensor and obtain more control over the hybridization process, a 2-piece sensor for riboflavin (2-piece-RB) was designed by coating the AuNPs directly with the aptamer. A set of particles (AuNP-DNA12) was coated with a 12-base sequence complementary to the first 12 bases of the 5′ end of the aptamer. Hybridization of the 2-piece sensor required a higher salt concentration, compared to the 3-piece. This difference is due to the fact that immobilization of the aptamer on the surface of the AuNPs required the particles to get closer to hybridize. As a consequence, a more effective screening of the charges was necessary. Figure 3A shows the melting curves for three different 2-piece-RB sensors obtained with different combinations of the DNA-AuNPs. Sharp curves were obtained when equimolar amounts or a slight excess of one of the AuNPs were used, contrary to the case when a large excess of one set of the particles was included. On the base of these results, the sensors were prepared in a 1:1 (2-piece-RB-1) or 1:0.75 (2-piece-RB-2) ratio of AuNP-Aptamer:AuNP-

DNA12. The influence of the ions used in the hybridization buffer was studied by letting the sensors hybridize in two different buffer systems at slightly different pH values. Both buffers produced aggregates with sharp melting curves, as shown in Figure 3B and C.

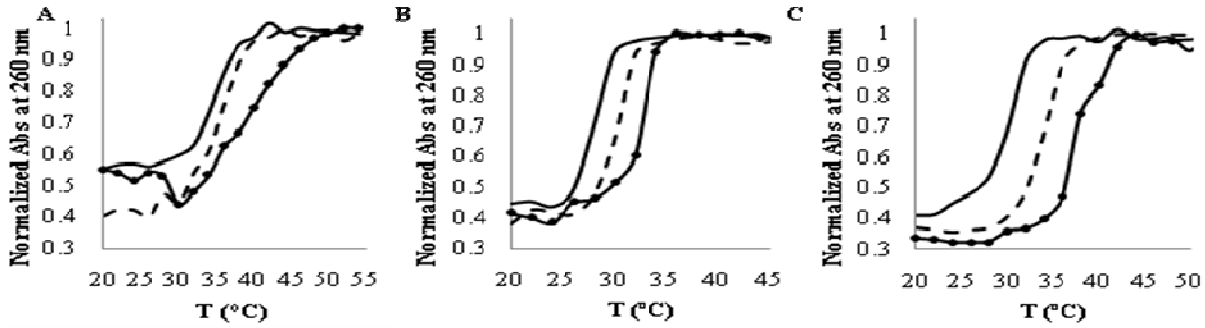

Figure 3. A) Melting curves of 2-piece-RB aggregates suspended in 25 mM tris acetate containing 400 mM NaCl, prepared with different AuNP-Aptamer:AuNP-DNA12 combinations: 1:0.75 (solid line), 1:1 (dashed line) and 1:2.5 (circled line). Melting curves of 2-piece-RB aggregates prepared with an equimolar mixture of DNA-AuNPs and hybridized in: B) 500 mM NaCl, 25 mM tris acetate, pH 8.2 and C) 400 mM KCl, 200 mM NaCl, pH 7.5. The melting curves were obtained at different concentrations of NaCl: B) 300 mM (solid line), 350 mM (dashed line) and 400 mM (circled line) and KCl/NaCl: C) 200/100 mM (solid line), 233/117 mM (dashed line) and 267/133 mM (circled line).

The sensor 2-piece-RB-2 was tested in response to riboflavin. Figure 4A shows the UV-vis response at different analyte concentrations for the sensor suspended in 350 mM NaCl at 23 °C. The suspensions change their color upon riboflavin addition at small concentrations. A further increase in the concentration of the analyte above 2.5 μM produced a fast increase in the intensity of the color of the suspensions. These results are shown quantitatively in Figure 4B with a plot of the corrected intensity of the absorption maximum at different analyte concentrations.

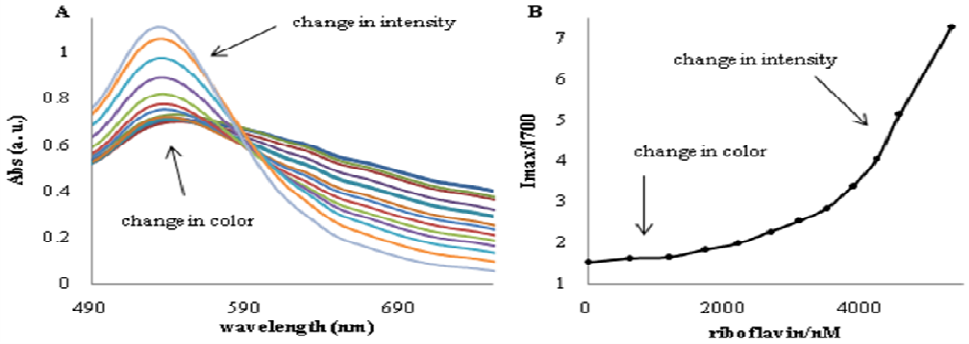

Figure 4. A) UV-vis response of 2-piece-RB-2 sensor suspended in 350 mM NaCl at 23°C to various concentrations of riboflavin, B) Plot of the corrected absorption maxima of 2-piece-RB sensor in detection buffer at different concentrations of riboflavin.

CONCLUSIONS

Gold nanoparticle-based sensors were designed by two approaches. A 3-piece sensor was prepared by coating AuNPs with short oligonucleotides complementary to parts of the aptamers for three analytes. The sensors showed sharp melting curves, suggesting a well-controlled assembly of the particles. A linear response of the sensor for theophylline over a wide range of concentrations was observed. The 3-piece sensor designed for ricin showed a superior sensitivity. A novel 2-piece sensor for riboflavin was designed in order to simplify the system and improve control over the assembly process. This design was based on linkage of the aptamer to the gold nanoparticles by a thiol group. The conditions necessary for the hybridization of this system were different than the 3-piece, a consequence of the immobilization of the aptamer on the surface of the particles. An equimolar mixture of the nanoparticles was observed to produce sensors with sharp melting curves that responded to riboflavin at different concentrations. This work offers a different option for the design of gold nanoparticle-based sensors with reasonable sensitivity over a wide range of analyte detection concentrations.

REFERENCES

1. N. L. Rosi, C. A. Mirkin, "Nanostructures in Biodiagnostics" *Chem. Rev.* **105**, 1547-1562 (2005).
2. J. Liu, J. Lu, "Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles" *Angew. Chem. Int. Ed.* **45**, 90-94 (2006).
3. J. Liu, J. Lu, "Preparation of aptamer-linked gold nanoparticles purple aggregates for colorimetric sensing of analytes" *Nature Protocols* **1**[1], 246-252 (2008).
4. S. A. Lynch, S. K. Desai, H. K. Saija, J. P. Gallivan, “A High-Throughput Screen for Synthetic Riboswitches Reveals Mechanistic Insights into Their Function”, *Chemistry and Biology* **14**, 173-184 (2007).
5. C. T. Lauhon, J. W. Szostak, “RNA Aptamers that Bind Flavin and Nicotinamide Redox Cofactors”, *J. Am. Chem. Soc.*, **117**, 1246-1257 (1995).
6. J. Tang, J. Xie, N. Shao, Y. Yan, “The DNA Aptamers that Specifically Recognize Ricin Toxin are Selected by Two *In Vitro* Selection Methods”, *Electrophoresis* **27**, 1303-1311 (2006).
7. R. Jin, G. Wu, Z. Li, C. A. Mirkin, G. C. Schatz, "What controls the melting properties of DNA-linked gold nanoparticle assemblies?" *J. Am. Chem. Soc.* **125**, 1643-1654 (2003).